Protonex™ Dyes

Ordering Information	*Storage Conditions*
Product Numbers: 21207, 21215 (1 mg)	Keep at -20 °C and desiccated, Avoid exposure to light

Biological Applications

AAT Bioquest's Protonex™ dyes demonstrated pH-dependent fluorescence. Unlike most of the existing fluorescent dyes that are more fluorescent at higher pH, acidic conditions enhance the fluorescence of Protonex™ dyes. The fluorescence of Protonex™ dyes increase as pH decreases from neutral to the acidic. The lack of fluorescence outside the cell eliminates the wash steps. Protonex™ dyes provide a powerful tool to monitor acidic cell compartments such as endosomes and lysosomes. Protonex™ dyes are non-fluorescent outside the cells, but fluoresce brightly in acidic compartments (such as phagosomes, lysosomes and endosomes). Those Protonex™ dyes enable the specific detection of cellular acidic compartments with reduced signal variability and improved accuracy for imaging or flow applications. Protonex™ Green has the spectral properties similar to those of FITC, and Protonex™ Red has the spectral properties similar to those of Texas Red, making the common filter set of FITC and Texas Red readily available to the assays of Protonex™ Green and red respectively.

Chemical and Physical Properties

Catalog Number	ROS Brite™ Dyes	Molecular Weight	Solvent	Excitation	Emission
21207	Protonex™ Red 600	984.03	DMSO	575 nm	597 nm
21215	Protonex™ Green 500	400.47	DMSO	443 nm	505 nm

Assay Protocol with Protonex™ Dyes

This protocol only provides a guideline, and should be modified according to your specific needs. Treat cells as desired before making the Protonex™ working solution.

1. Prepare a 1 to 10 mM Protonex™stock solution in DMSO. Make 0.1 to 10 µM working solution by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS) or buffer of your choice.
2. Treat cells as desired.
3. Incubate the cells with Protonex™ working solution for 15min to 2 hours at 37 ºC.
4. Replace the dye-loading solution with HHBS buffer.
5. Analyze the cells with a proper fluorescence instrument fitted with the correct filter set

References

1. Sarantis H, Grinstein S. (2012) Monitoring phospholipid dynamics during phagocytosis: application of genetically-encoded fluorescent probes. Methods Cell Biol, 108, 429.
2. Schreiner L, Huber-Lang M, Weiss ME, Hohmann H, Schmolz M, Schneider EM. (2011) Phagocytosis and digestion of pH-sensitive fluorescent dye (Eos-FP) transfected E. coli in whole blood assays from patients with severe sepsis and septic shock. J Cell Commun Signal, 5, 135.
3. Leclerc L, Boudard D, Pourchez J, Forest V, Sabido O, Bin V, Palle S, Grosseau P, Bernache D, Cottier M. (2010) Quantification of microsized fluorescent particles phagocytosis to a better knowledge of toxicity mechanisms. Inhal Toxicol, 22, 1091.
4. Flannagan RS, Grinstein S. (2010) The application of fluorescent probes for the analysis of lipid dynamics during phagocytosis. Methods Mol Biol, 591, 121.

Disclaimer: *This product is for research use only and is not intended for therapeutic or diagnostic applications.*